Mutations in CYP17 and risk of early-onset breast cancer

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Australian Breast Cancer Family Study

Devised 1989-1991

Funded by NHMRC

Established 1992

Funded by NHMRC, VHPF and NSWCC

Expanded 1995-2000

Funded by NIH (USA) as part of a Cooperative Breast Cancer Family Registry

Case-Control-Family Design

Hopper, Bishop & Easton, *Lancet* 2005

- **Population-based sampling**
  Cases (Vic & NSW Cancer Registries)
  Controls (Government Electoral Rolls)
- **Epidemiological data (by questionnaire)**
- **Genetic and molecular (blood, tissue)**

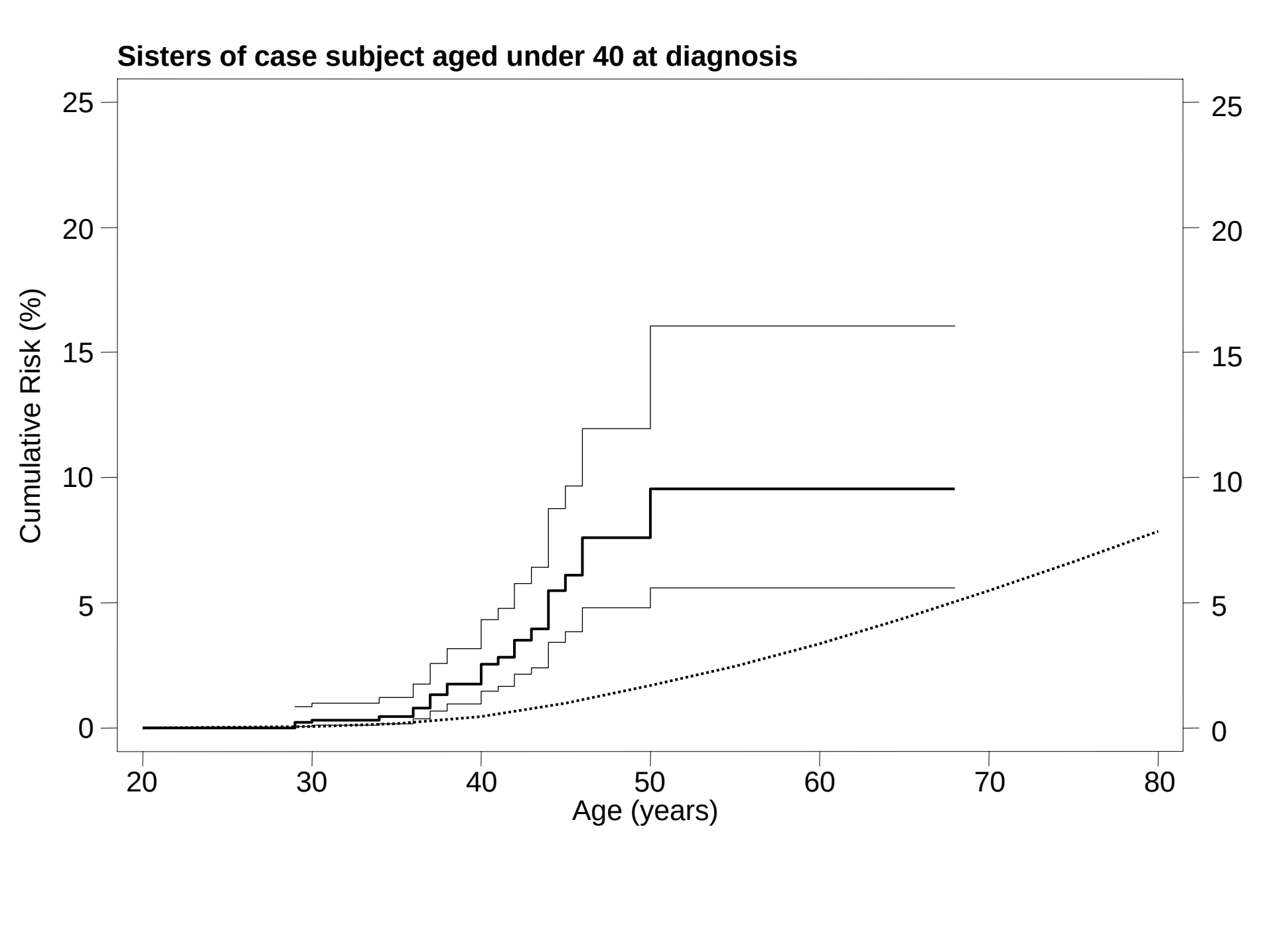
Data collected the same way for cases, controls, and relatives

Risks to Relatives of Cases

Dite et al., *JNCI* 2003

- **857 diagnosed before the age of 40**
- **367 diagnosed when aged 40-49**
- **355 diagnosed when aged 50-59**

Breast cancer risk in relatives, by age at diagnosis in case


Sisters of case subject aged under 40 at diagnosis
Cumulative Risk (%)
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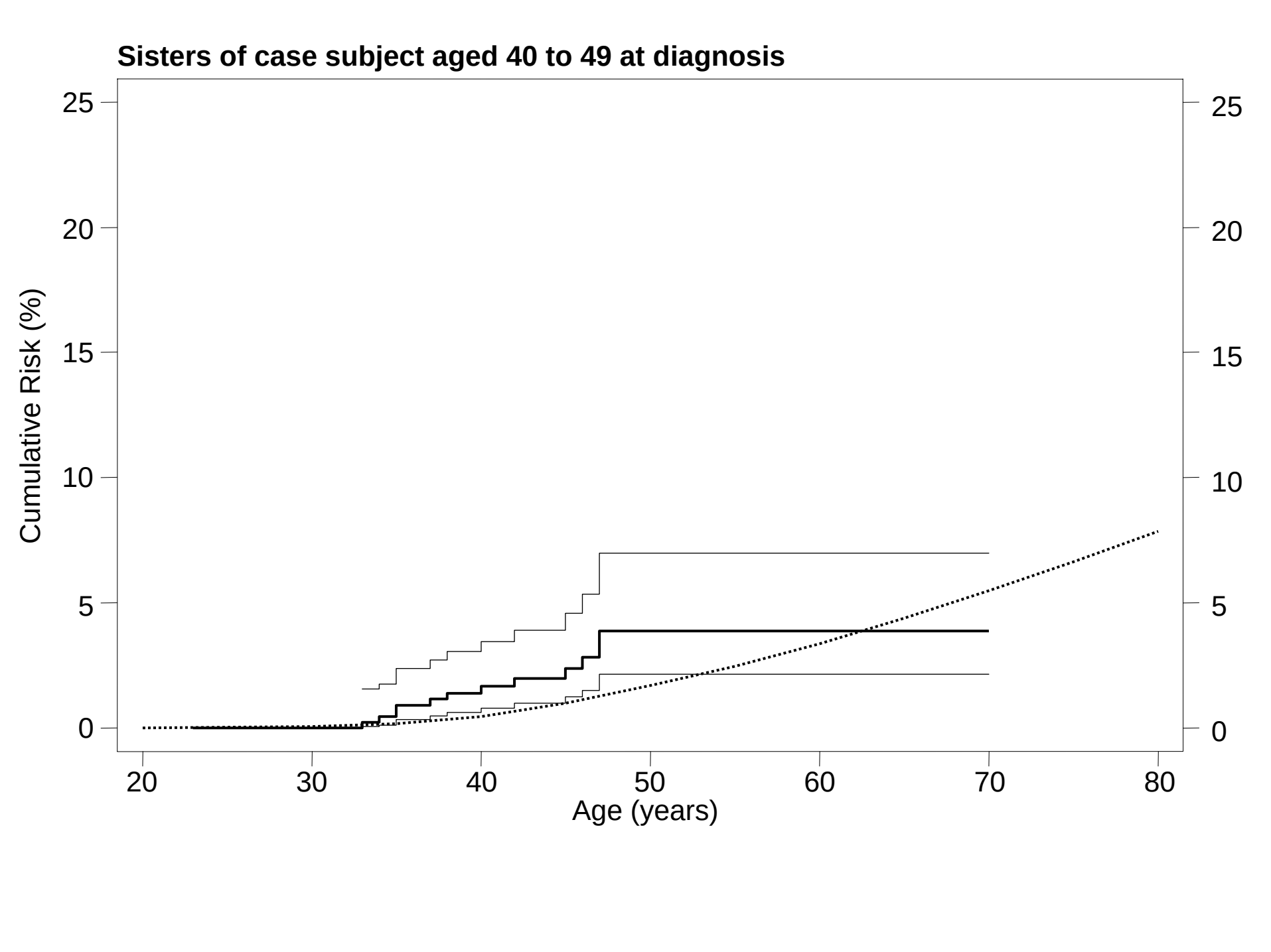
Age (years)


Sisters of case subject aged 40 to 49 at diagnosis
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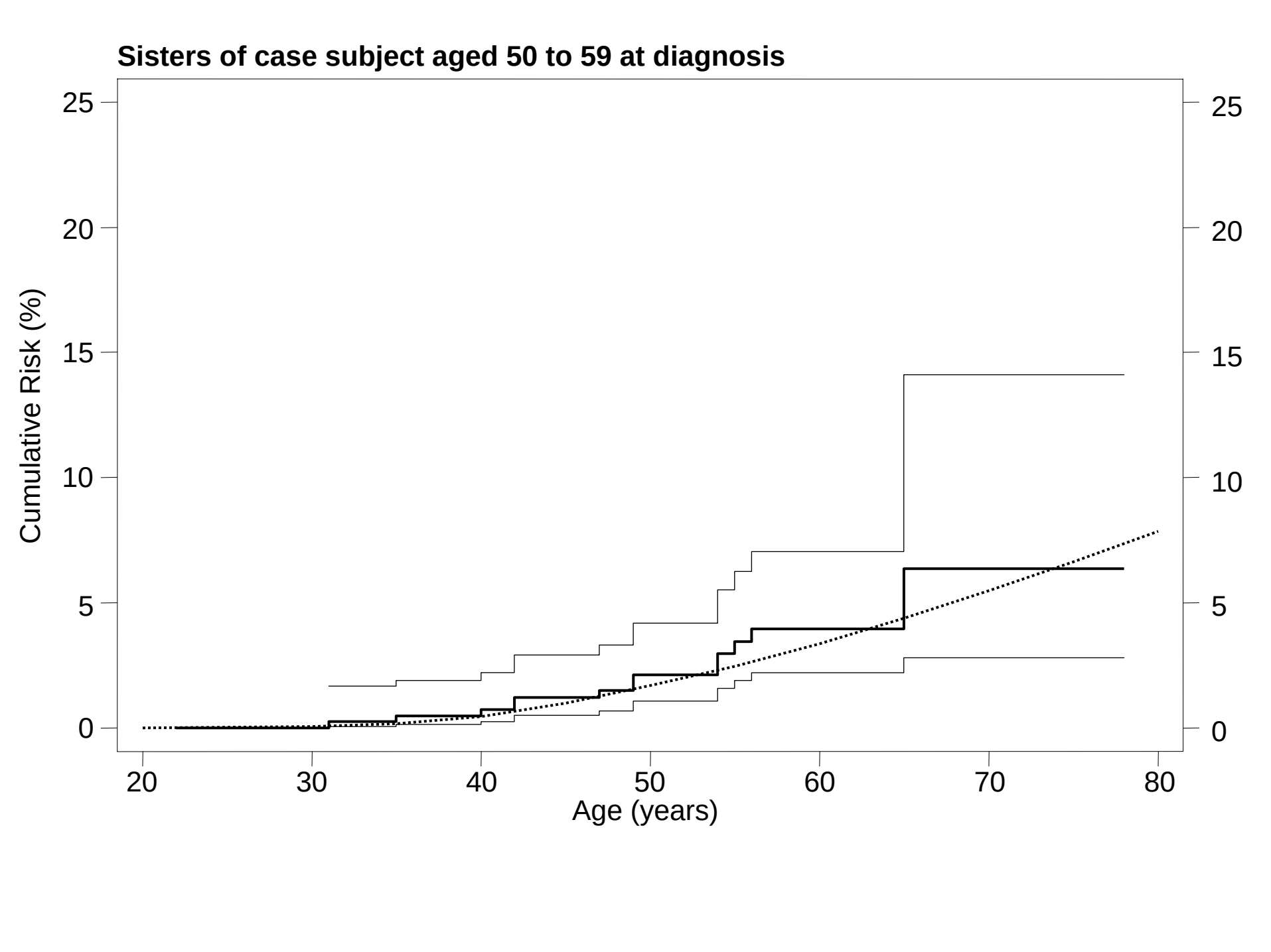
Sisters of case subject aged 50 to 59 at diagnosis
Cumulative Risk (%)
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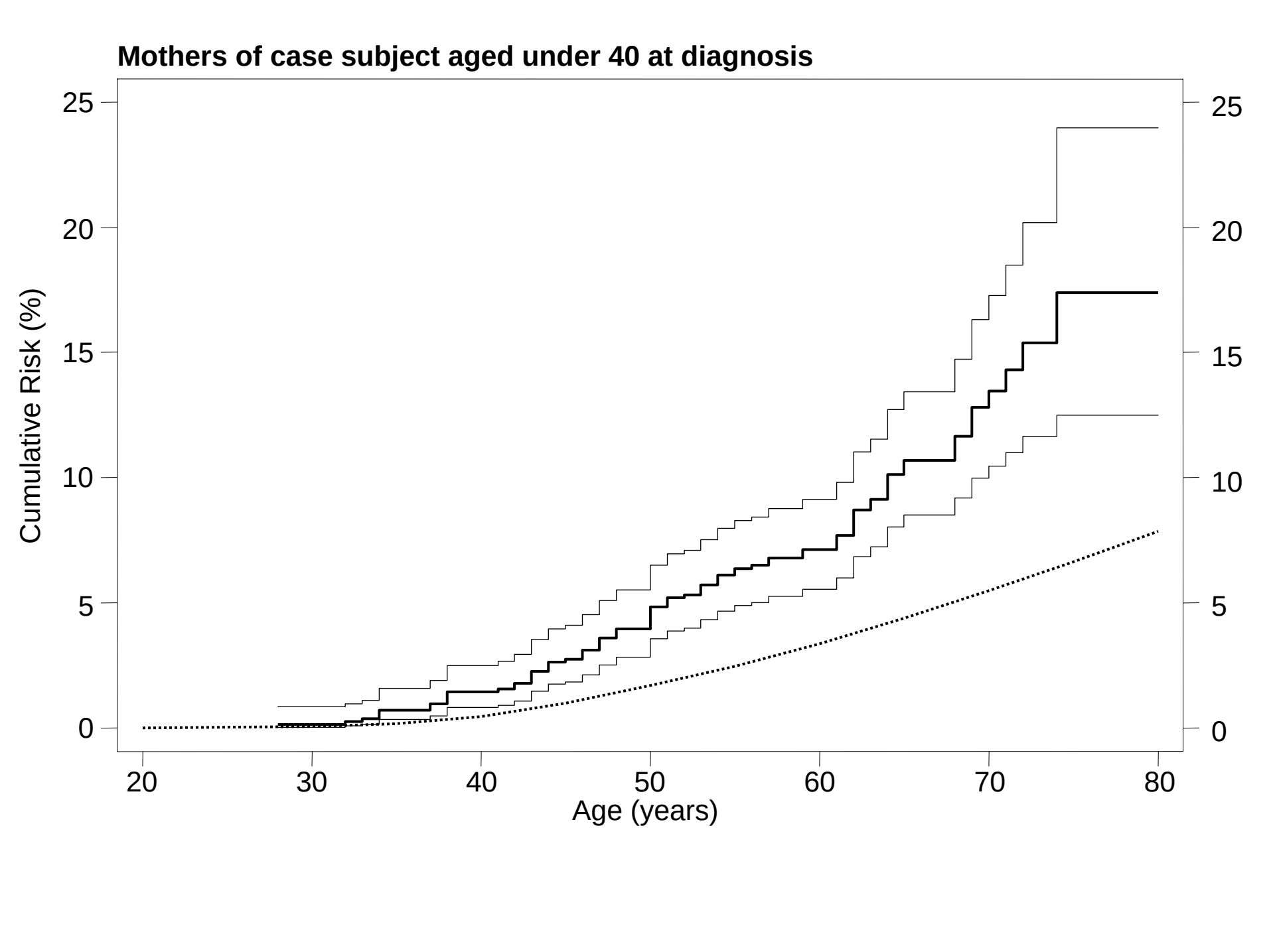
Age (years)


Mothers of case subject aged under 40 at diagnosis
Cumulative Risk (%)
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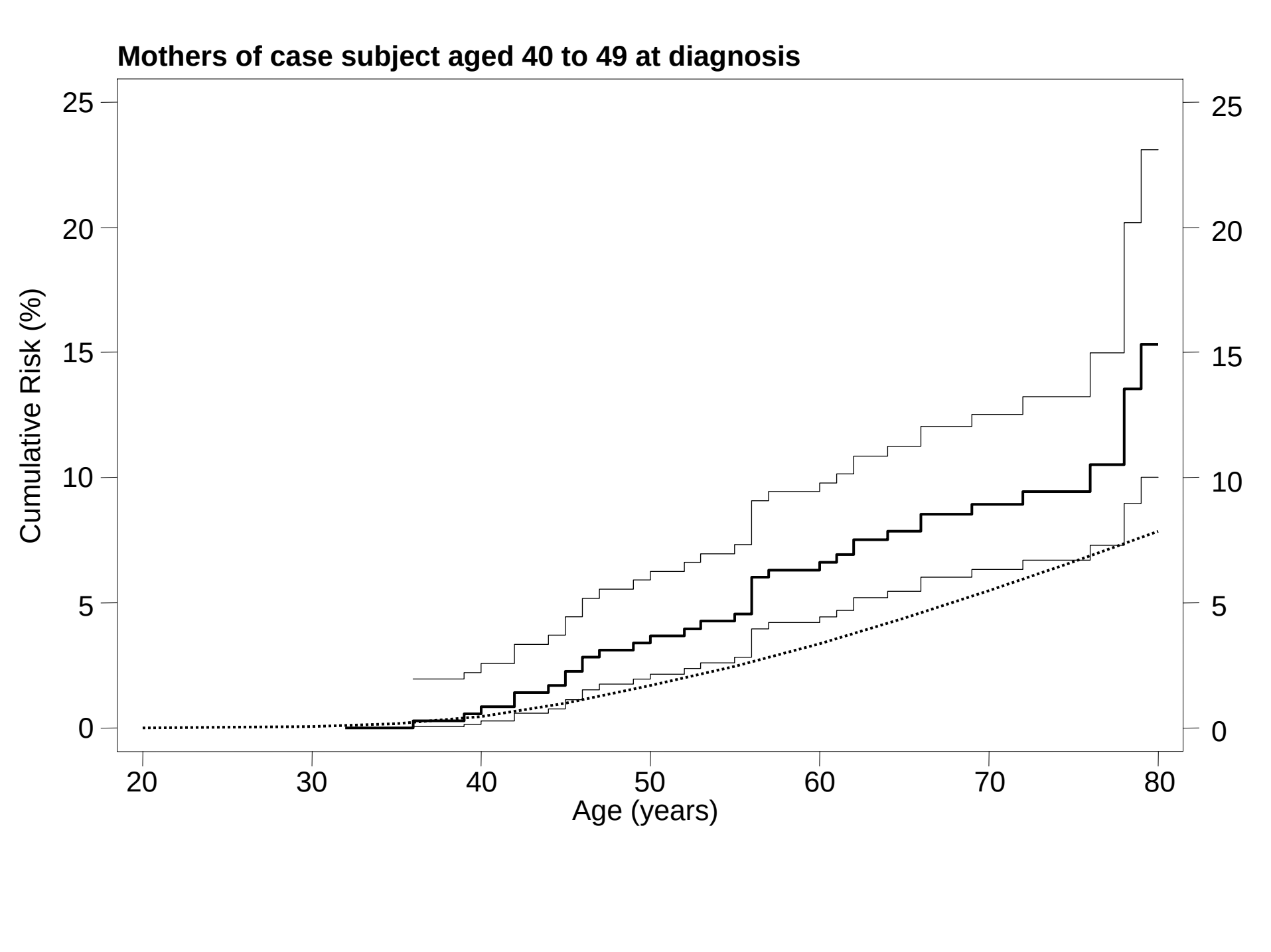
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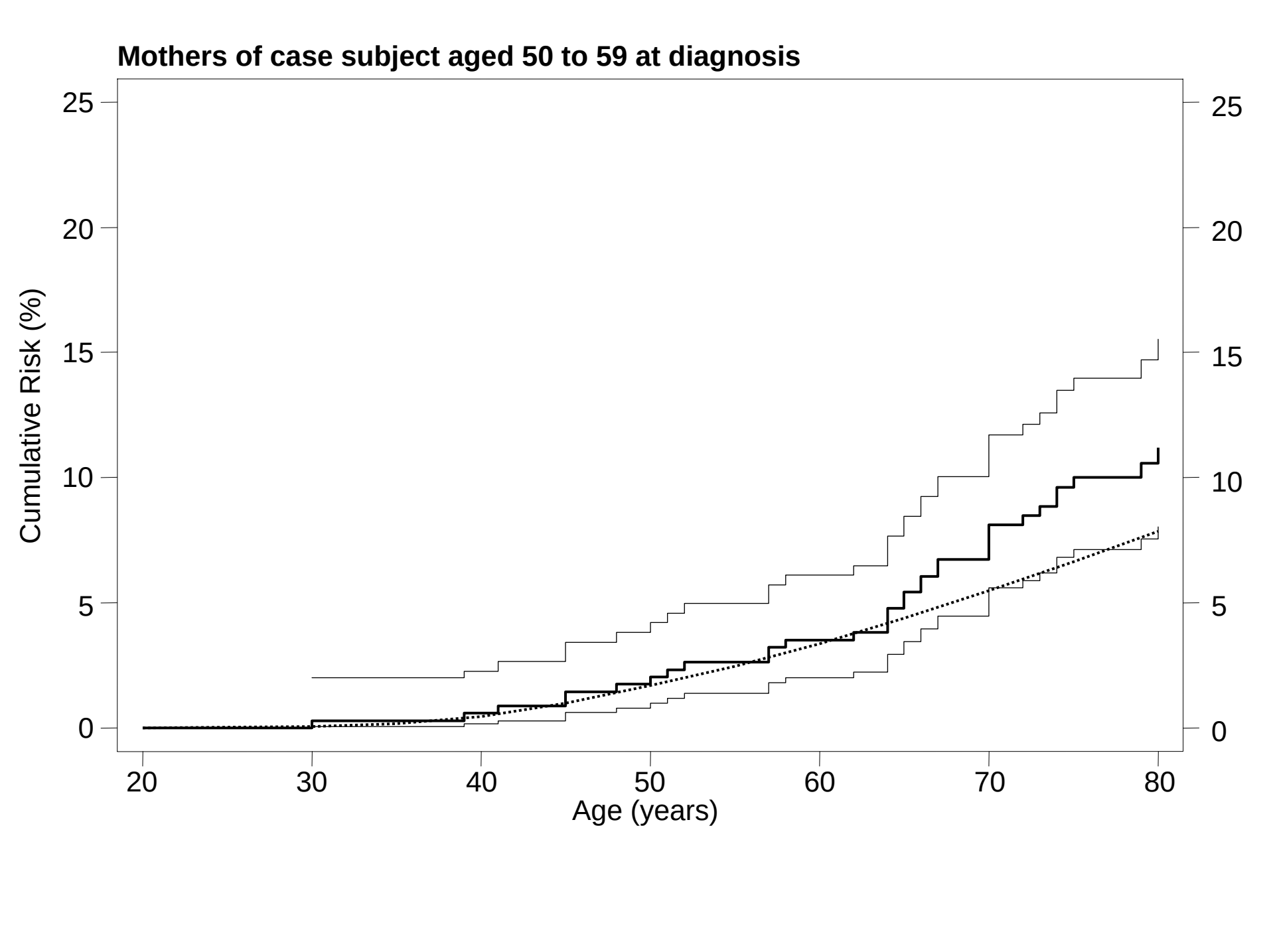
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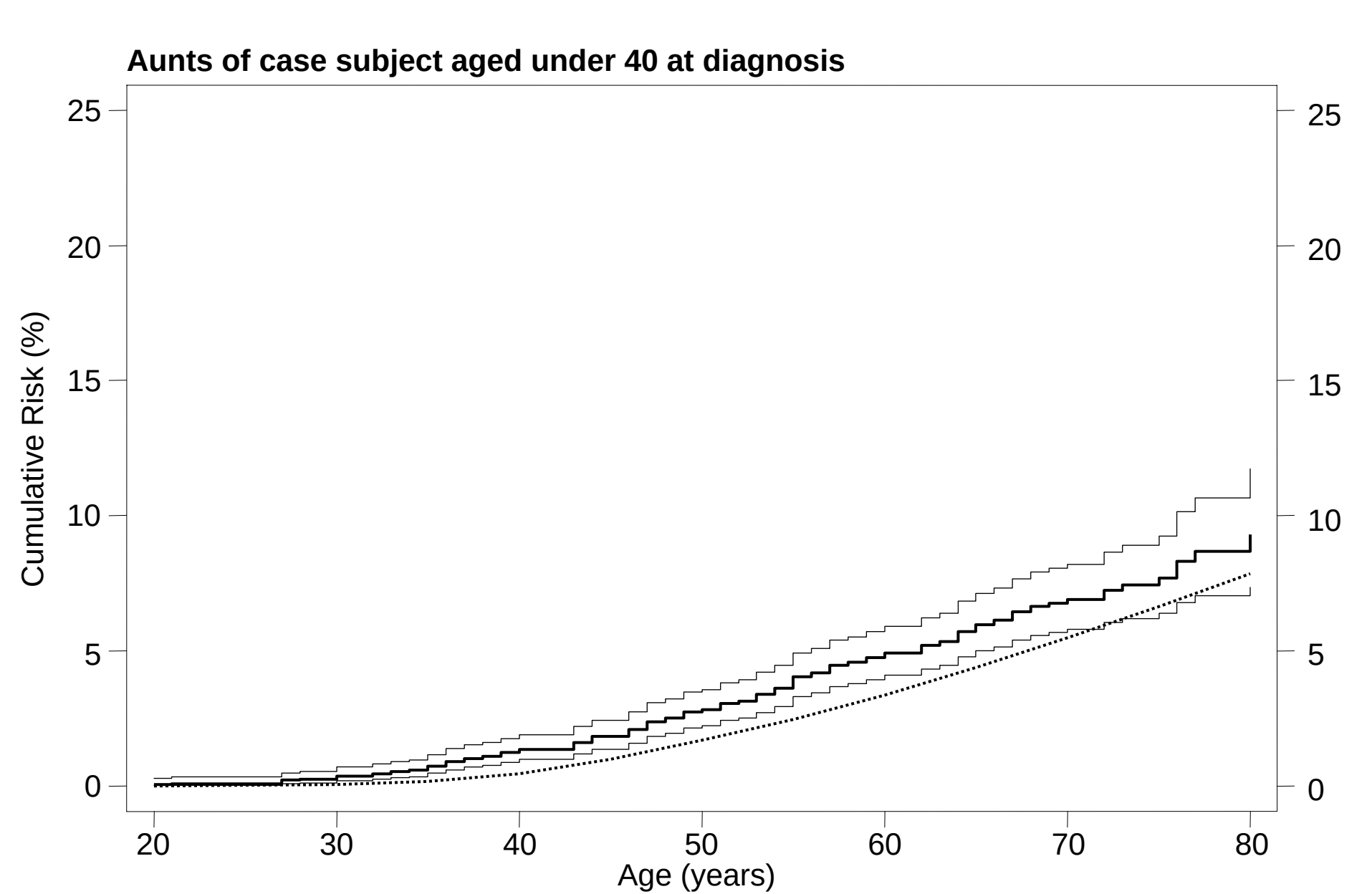
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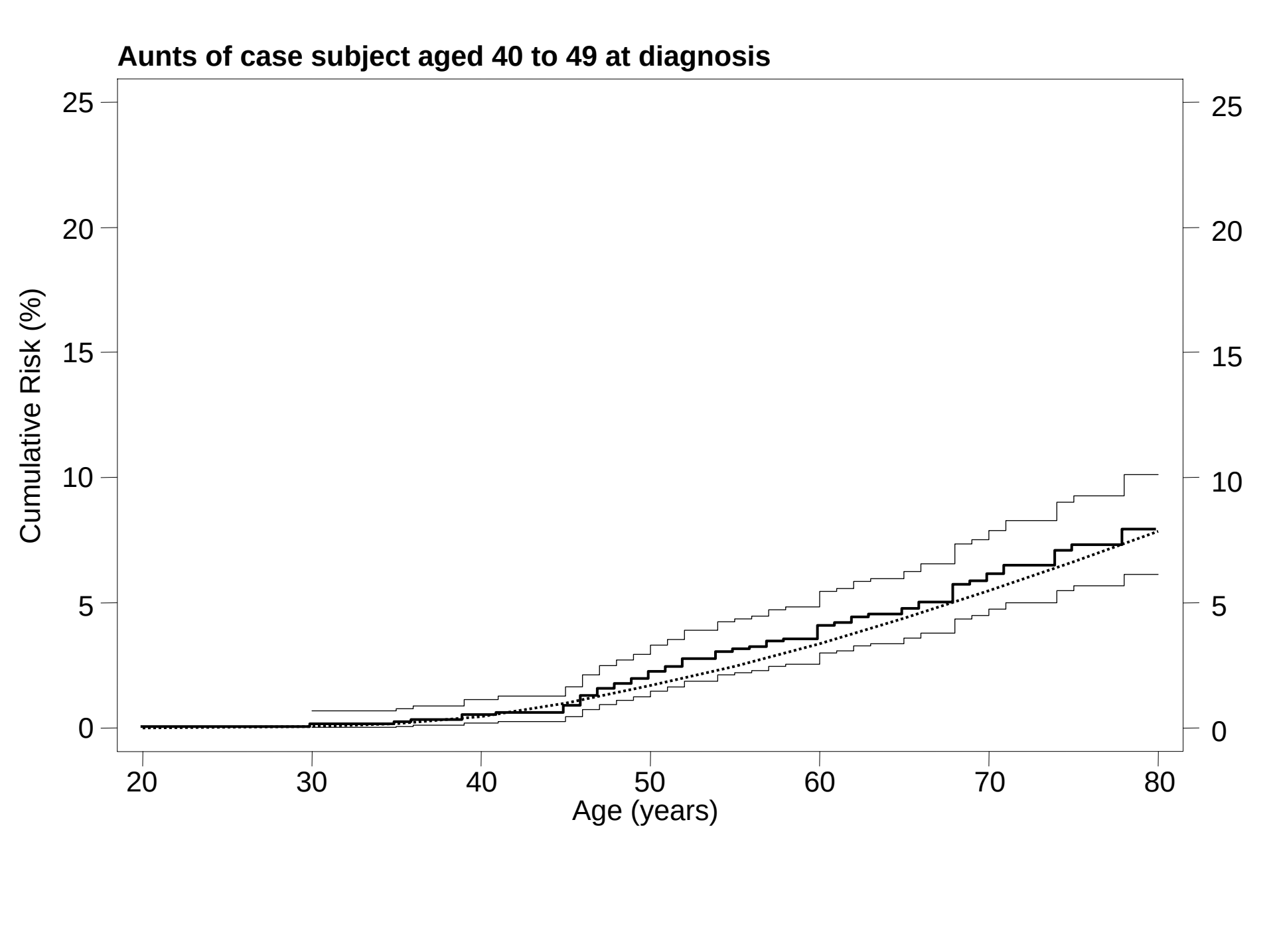
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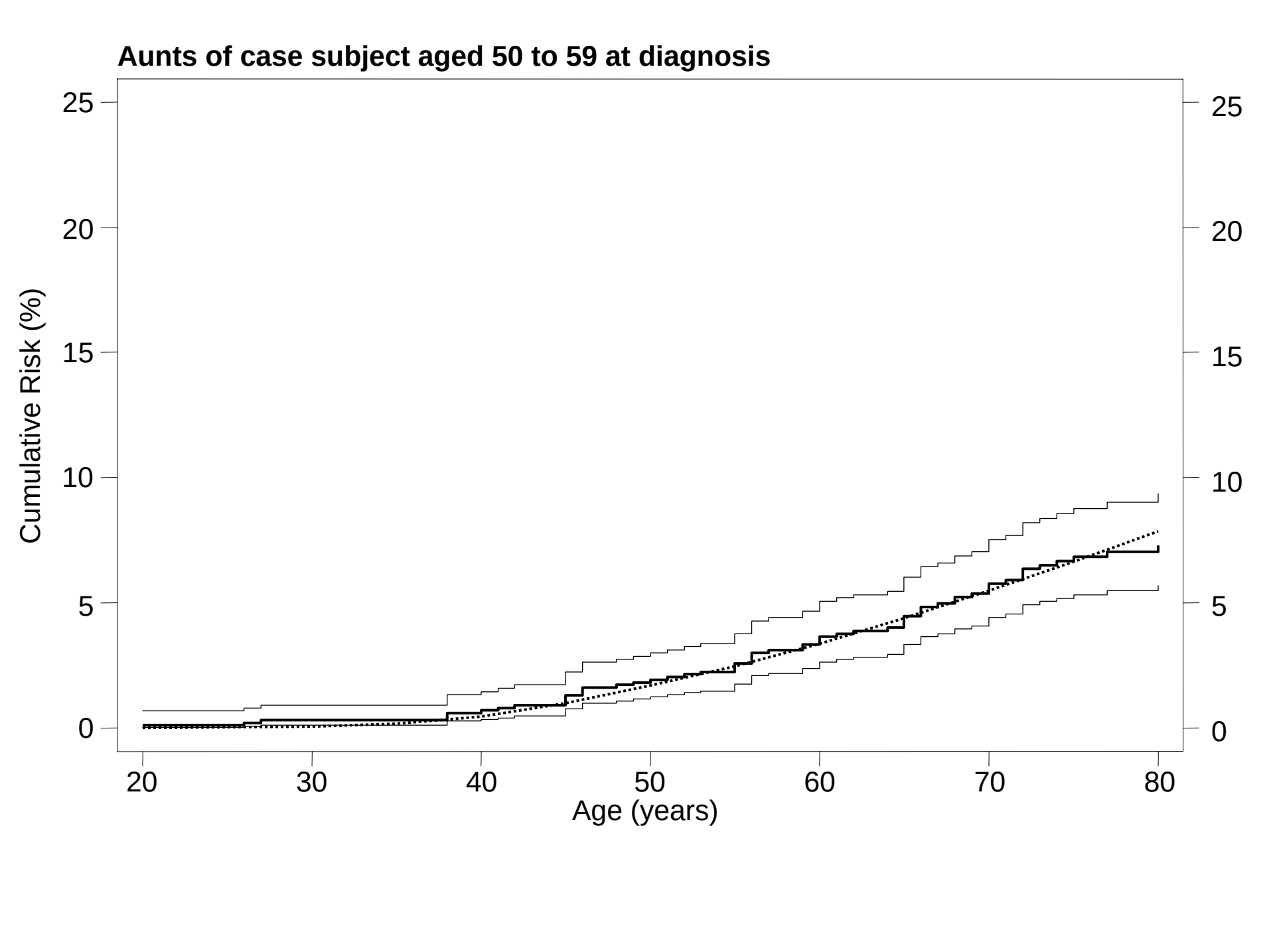
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Age (years)

BRCA1 & BRCA2 Mutation Testing

Manual sequencing of coding regions (and flanking intronic regions)

Protein Truncation Test exon 11 *BRCA1* & exons 10, 11 and 27 *BRCA2*

Test for Duplication exon 13 *BRCA1*

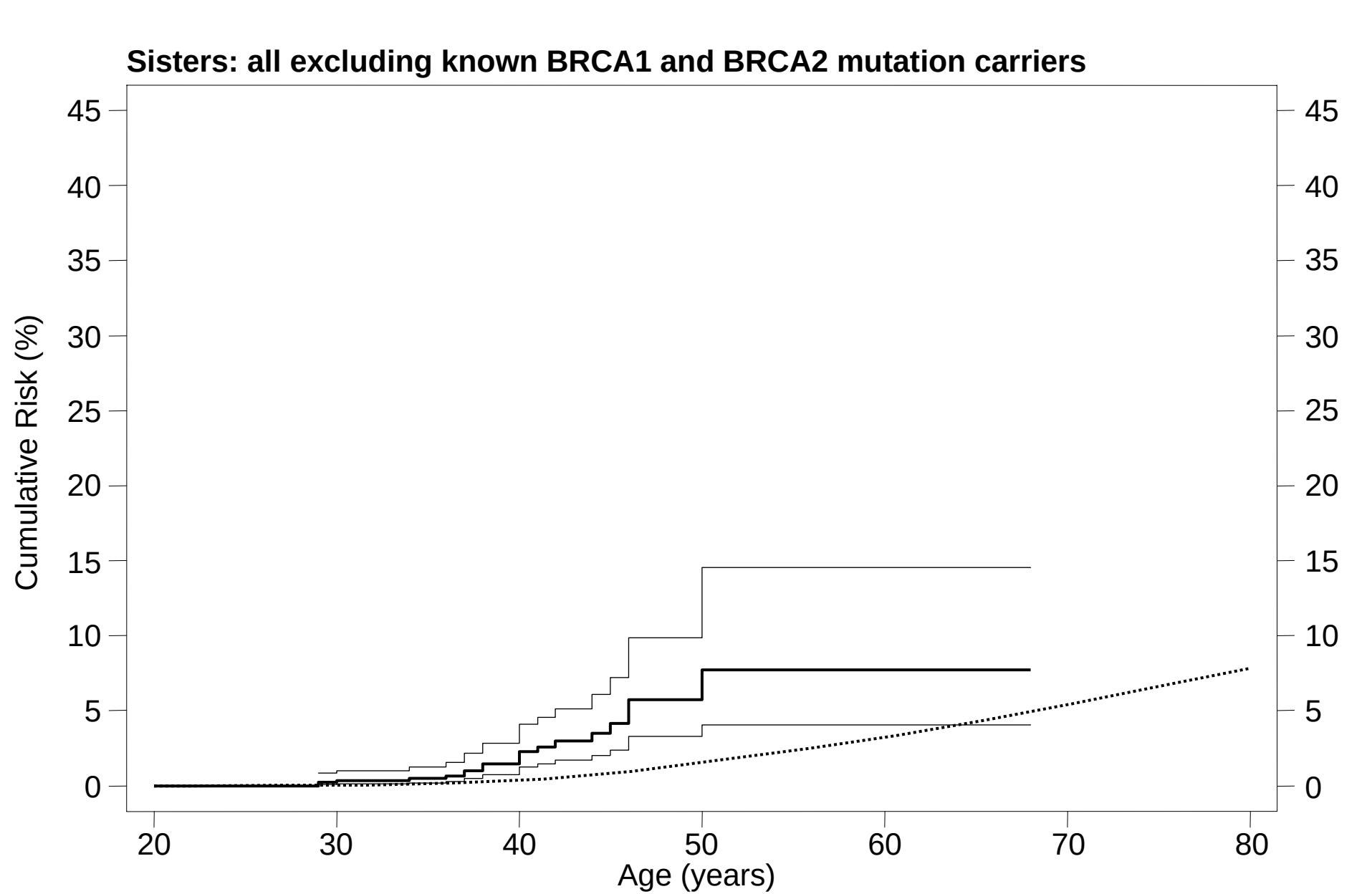
Multiplex Ligation-Dependent Probe Amplification (MLPA) *BRCA1*

Tested for *BRCA1* promoter mutations

BRCA Testing by *Myriad Genetics*

Excluding *BRCA1* & *BRCA2* Mutation Carriers

- Extensive mutation testing in case probands diagnosed before age 40
- 40 carriers (20 *BRCA1*, 20 *BRCA2*)
- Subdividing relatives by having one or more affected first-degree relatives, other than the (case) proband


Sisters: all excluding known BRCA1 and BRCA2 mutation carriers
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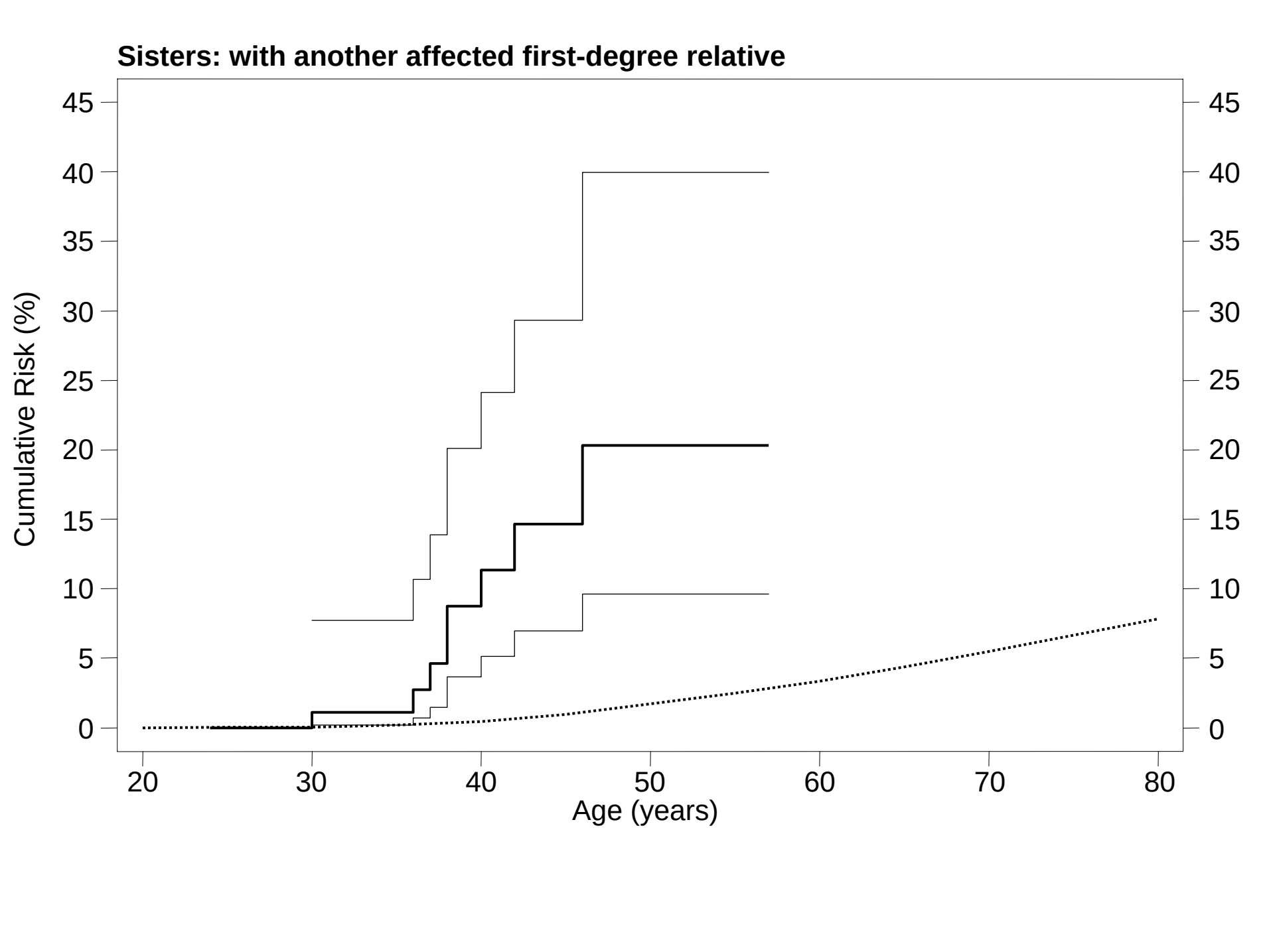
Sisters: with another affected first-degree relative
Cumulative Risk (%)
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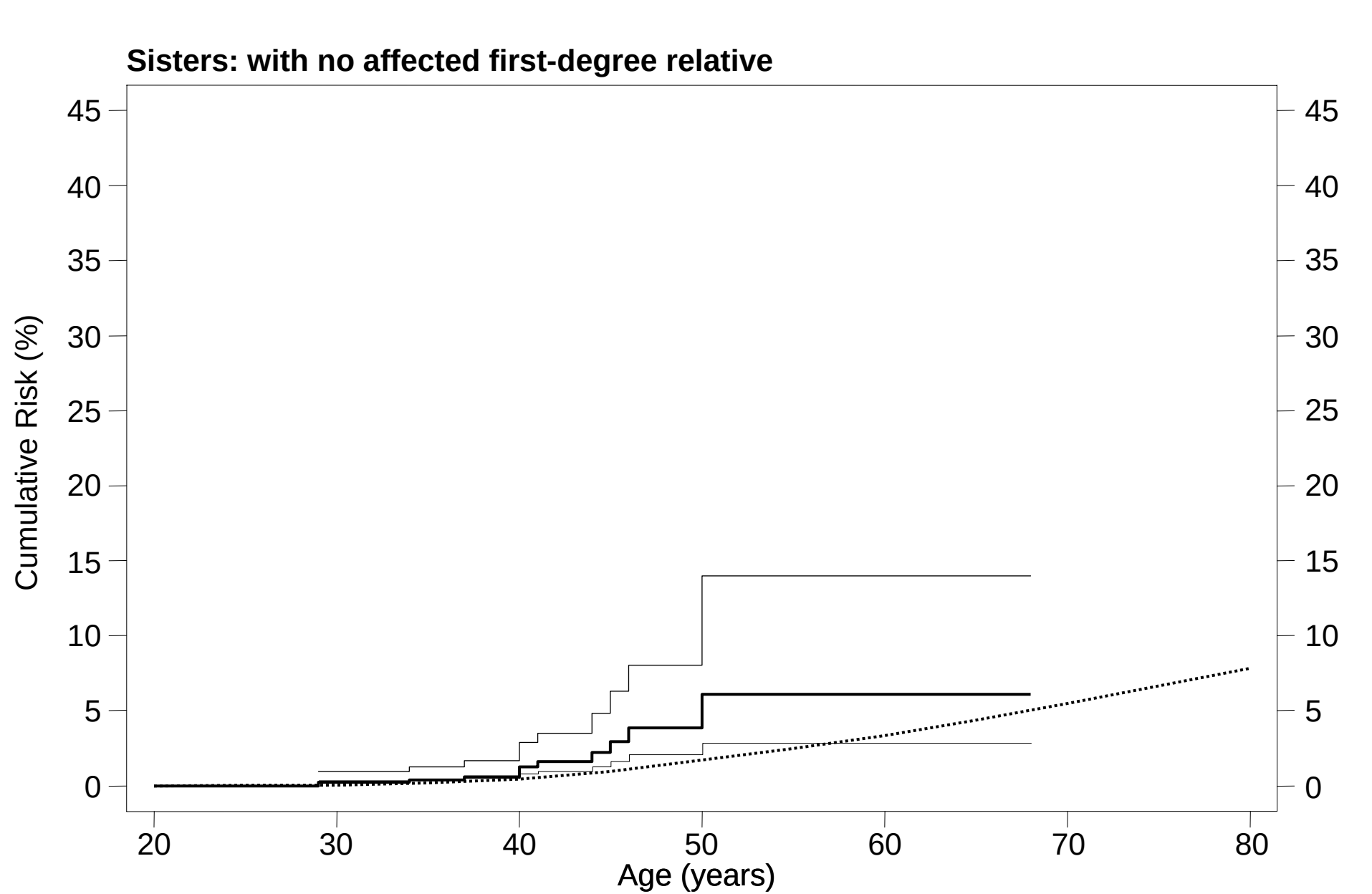
Sisters: with no affected first-degree relative
Cumulative Risk (%)
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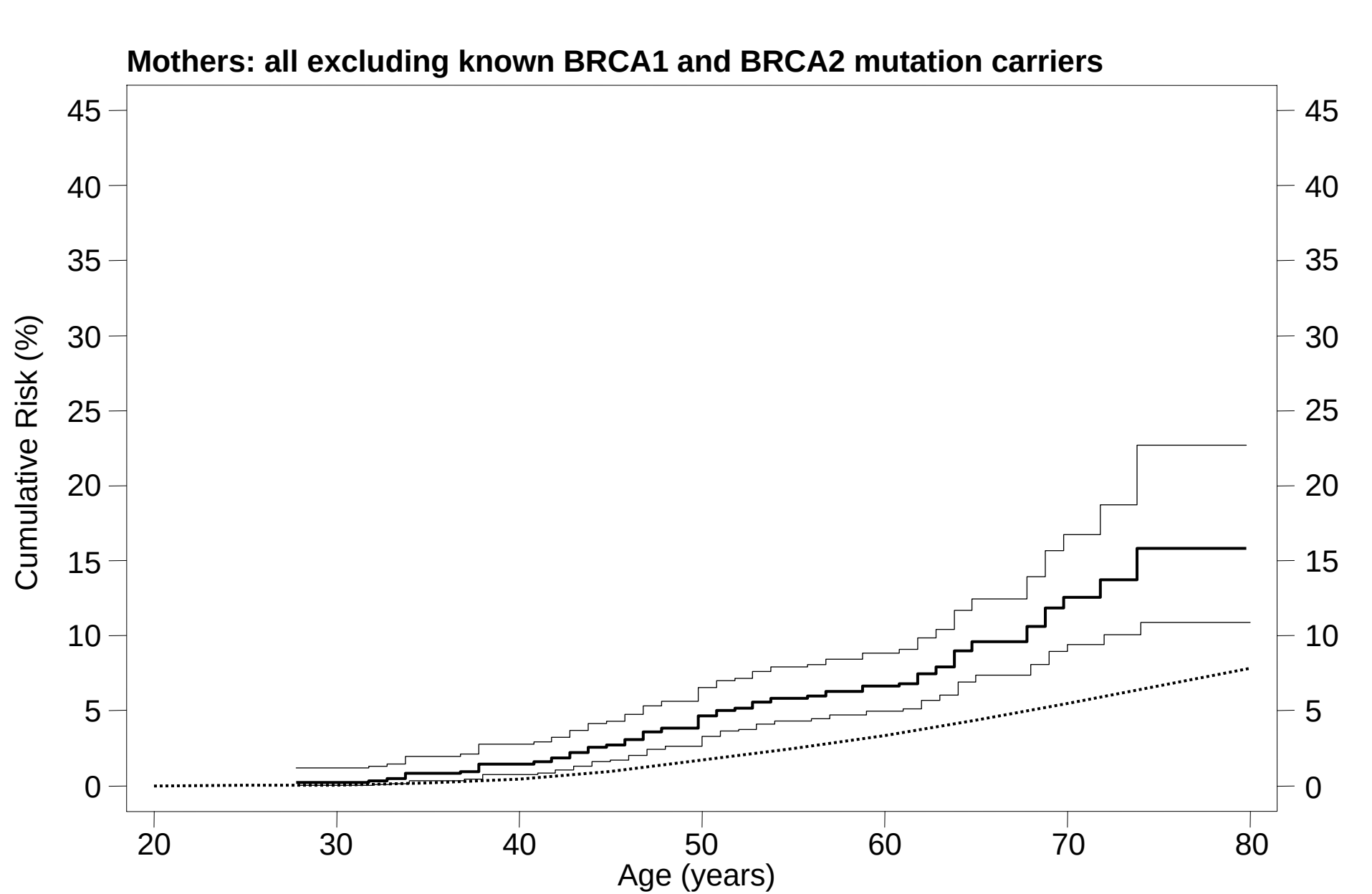
Age (years)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Cumulative Risk (%)
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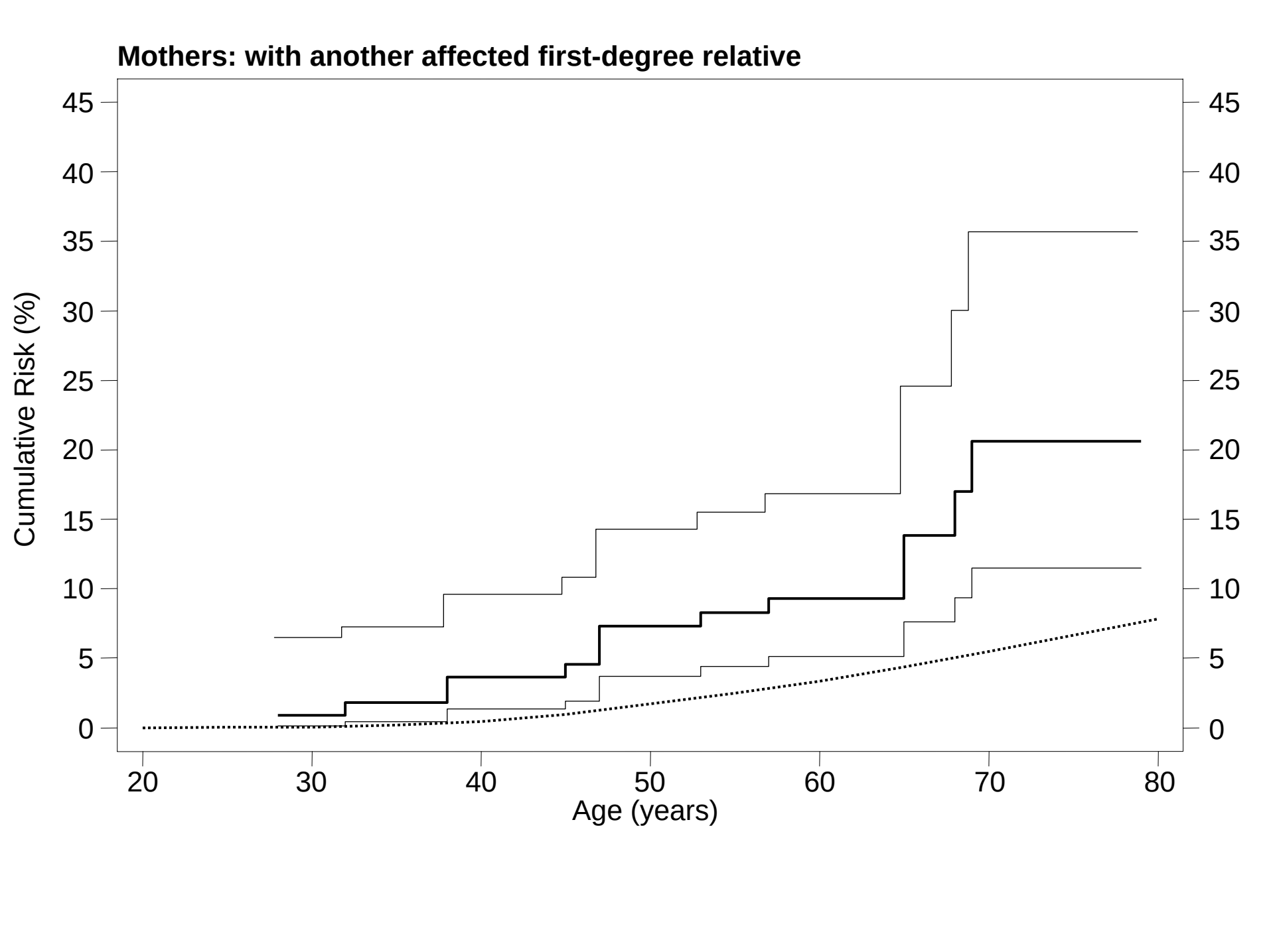
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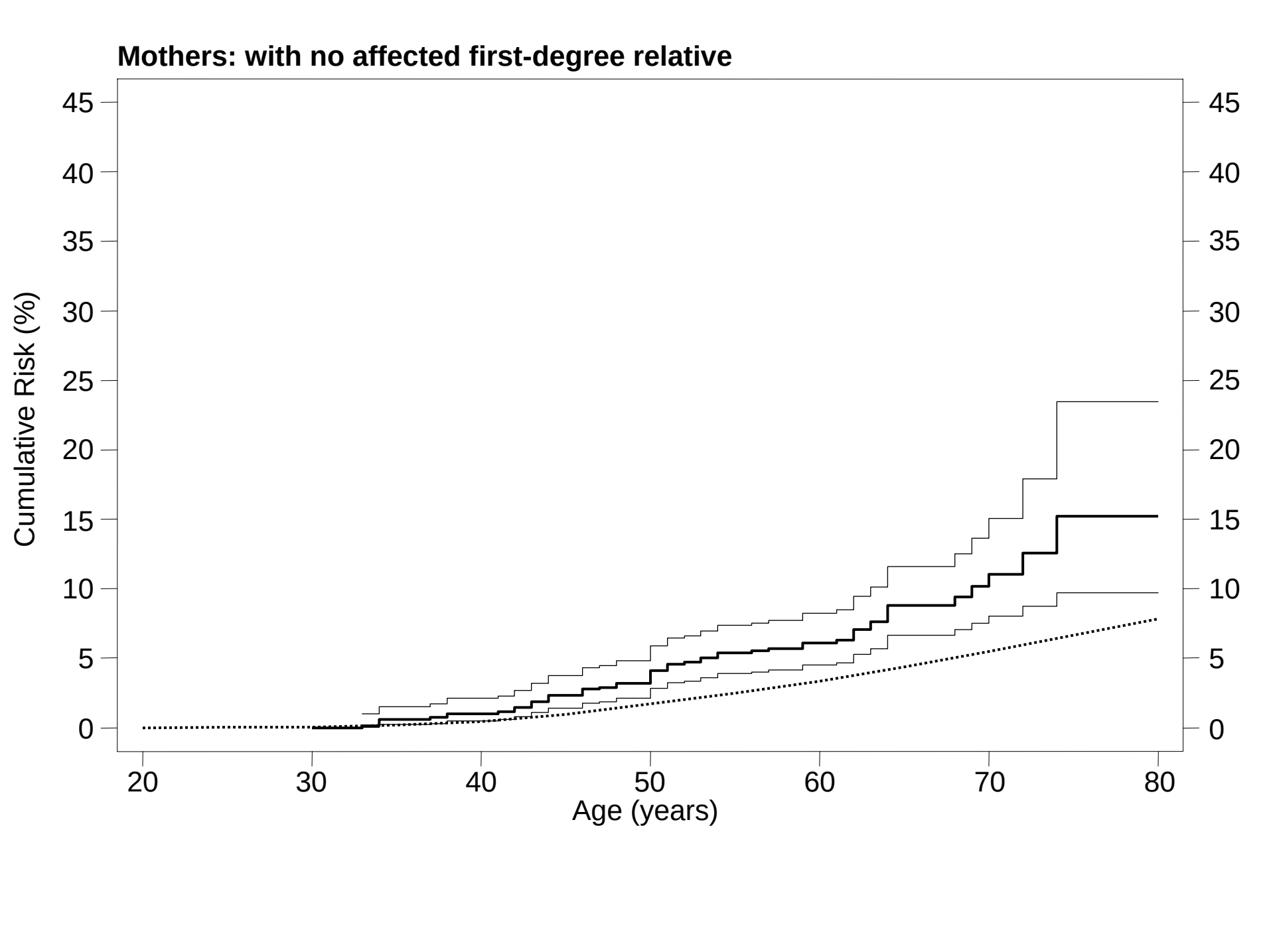
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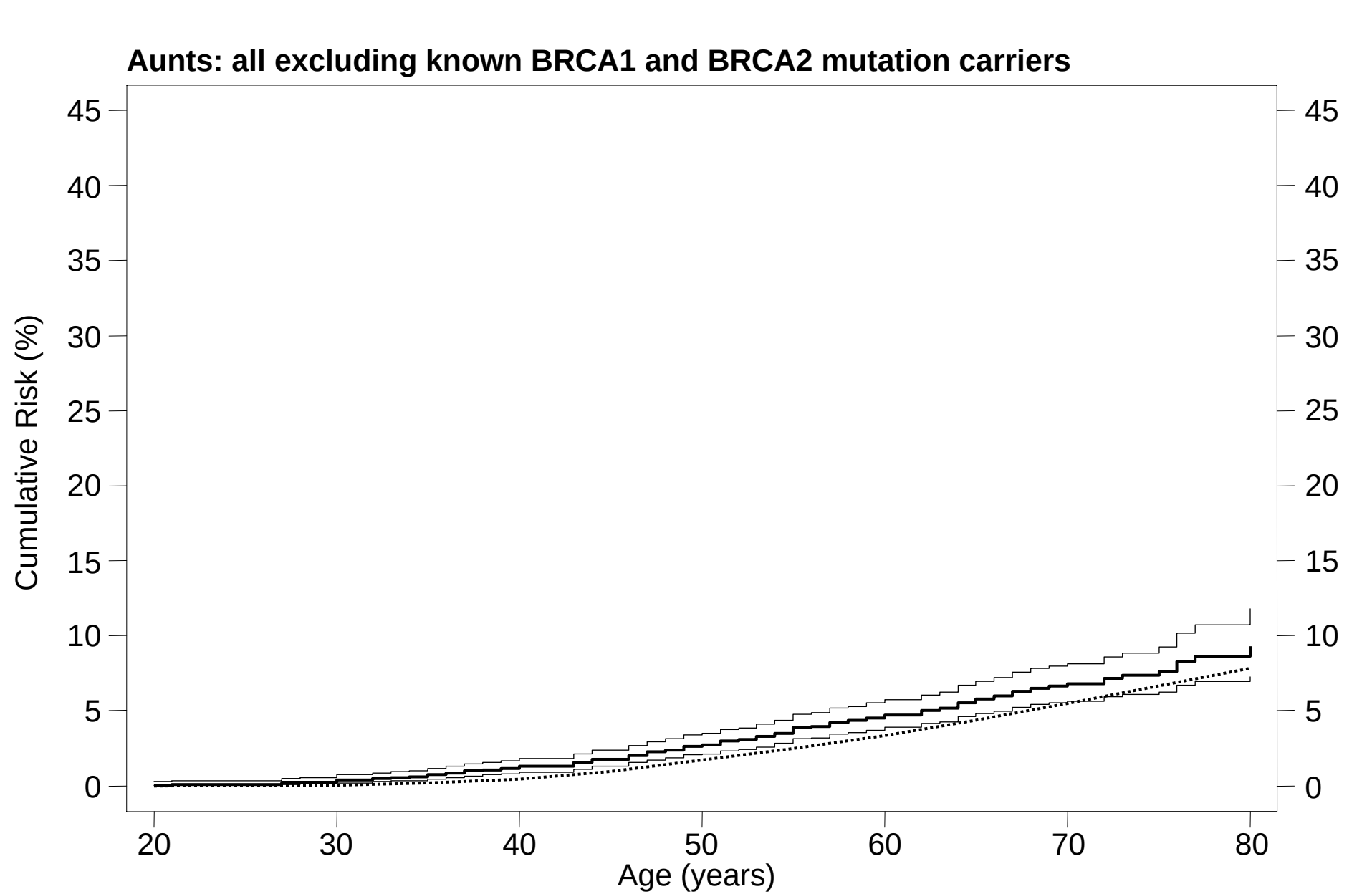
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Cumulative Risk (%)
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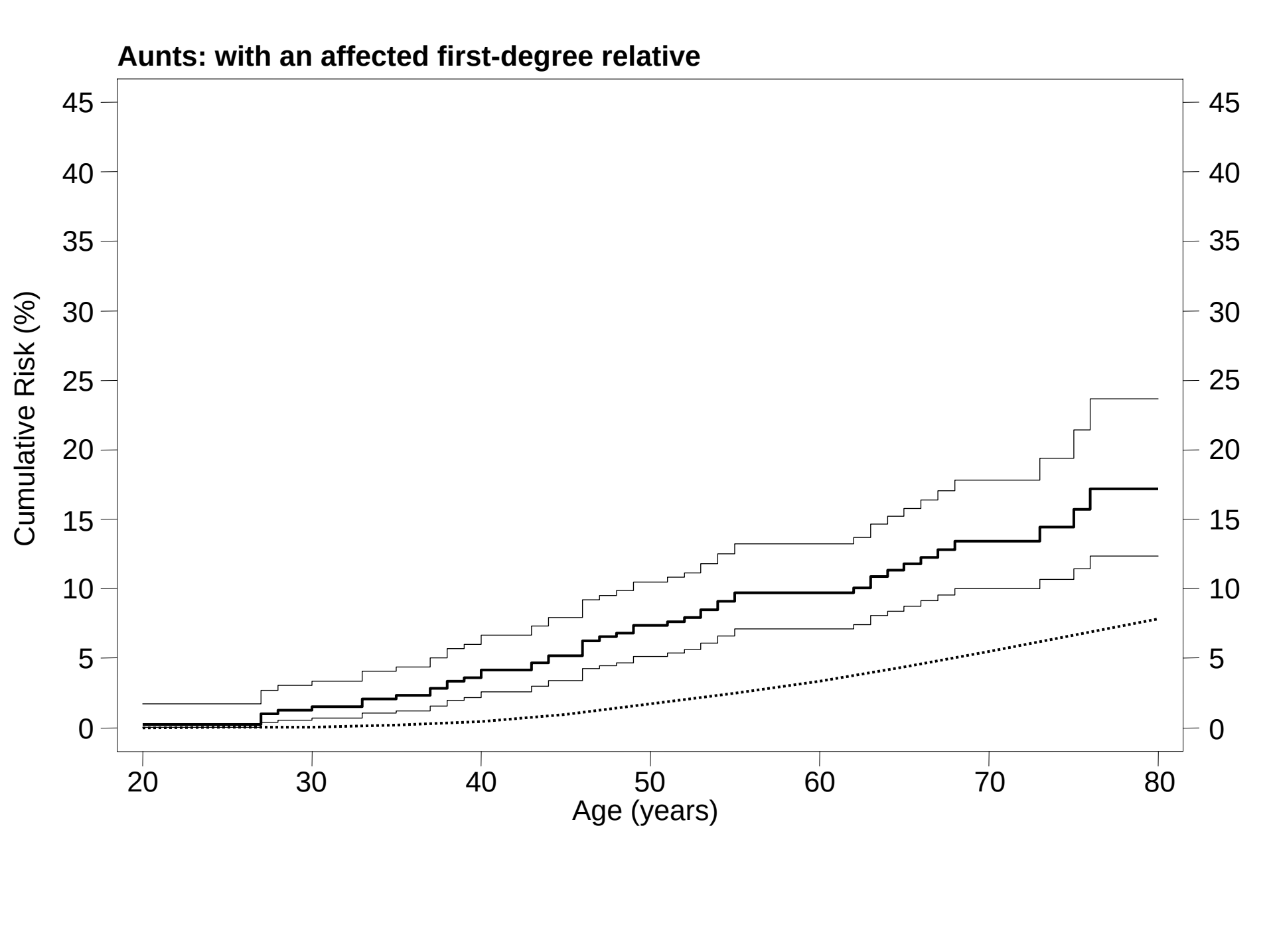
Age (years)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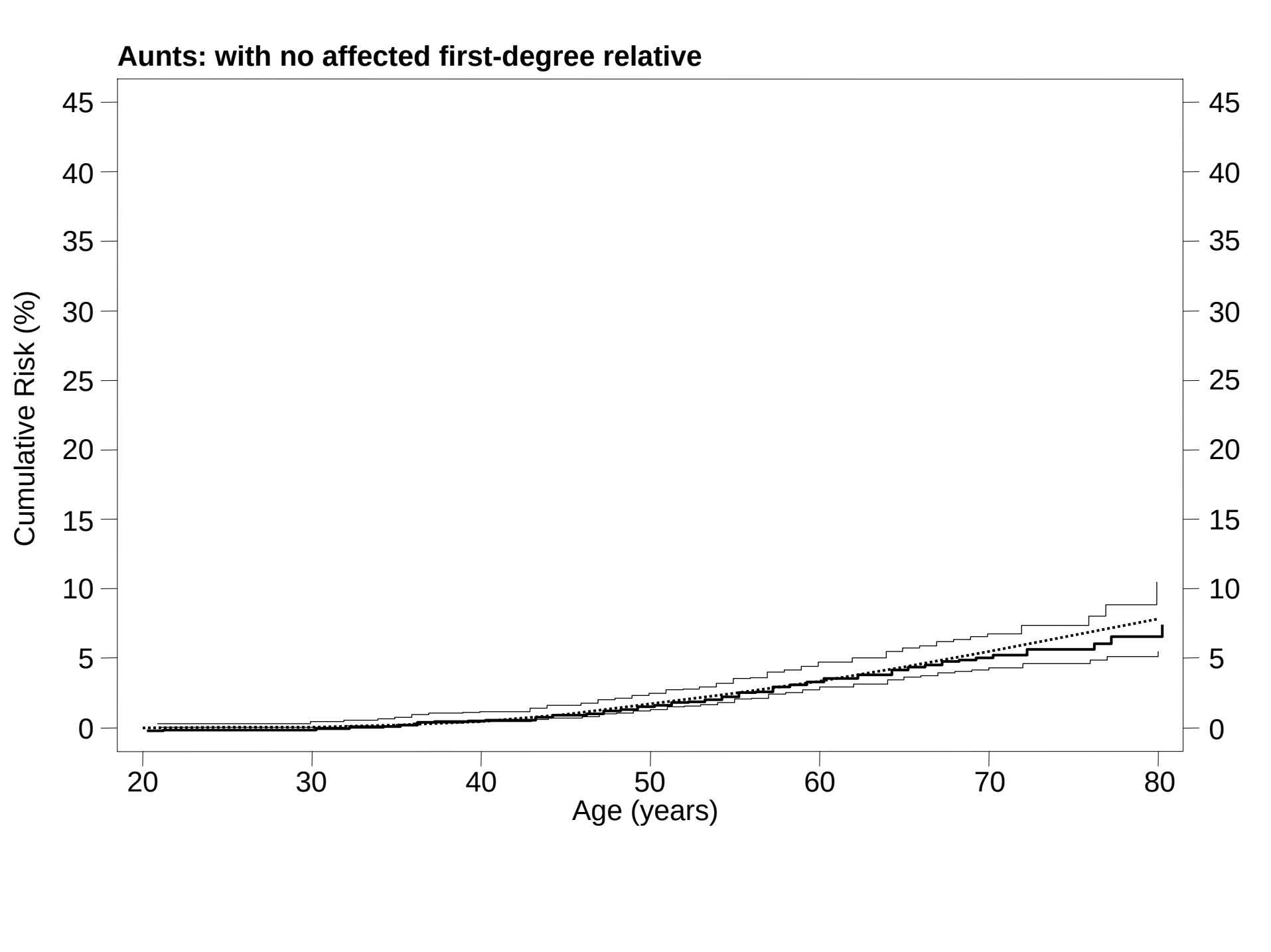
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Cumulative Risk (%)
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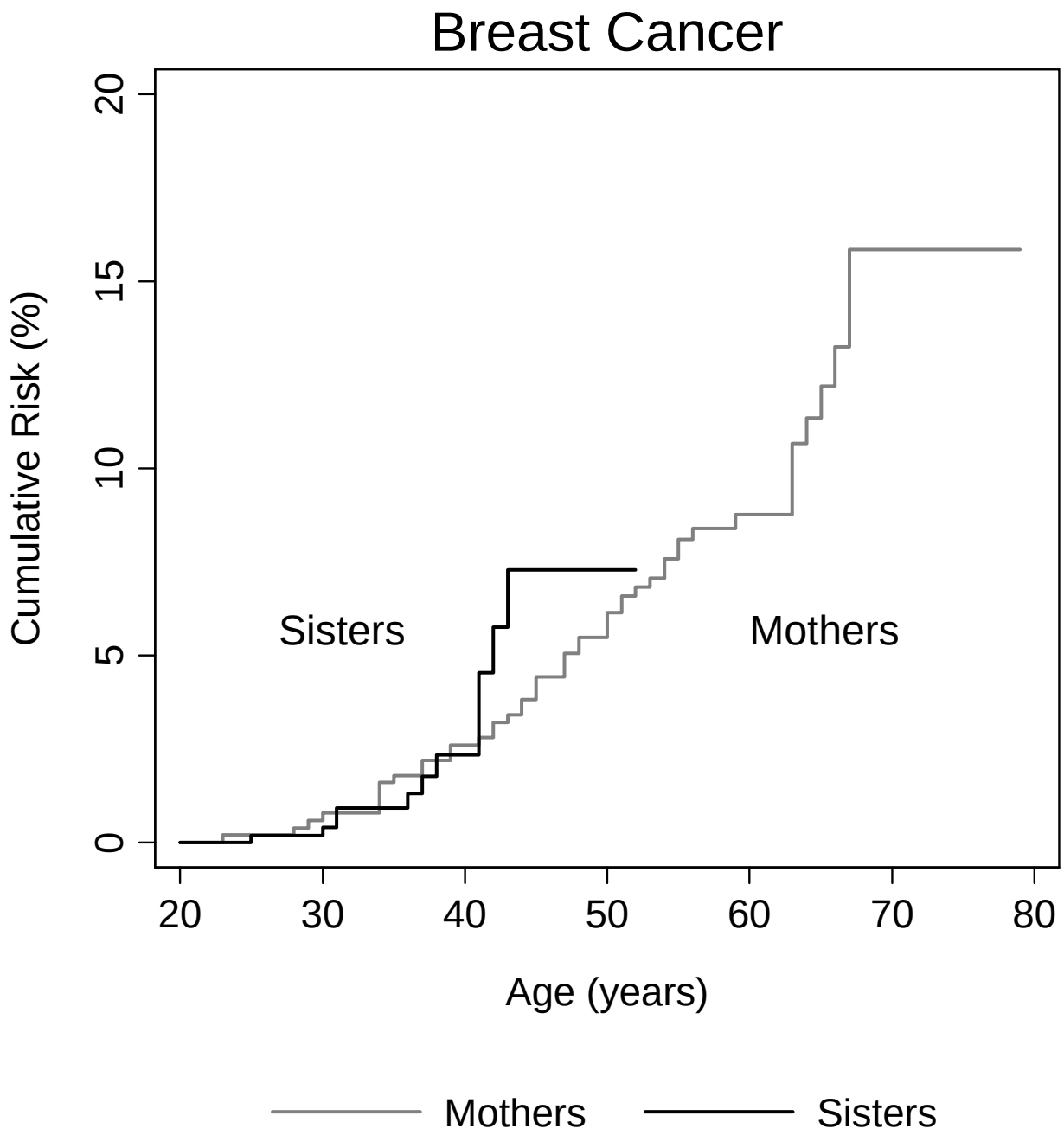
Age (years)

Risks to relatives of women with breast cancer before age 35

Dite et al., 2006

- **Australian, Nth California and Ontario population-based Breast CFR cases**
- **Unselected for family history**
- **Breast cancer risks for sisters and mothers**
- **Before and after excluding relatives of cases found to carry a mutation in *BRCA1* or *BRCA2***


Breast Cancer
Cumulative Risk (%)
0
5
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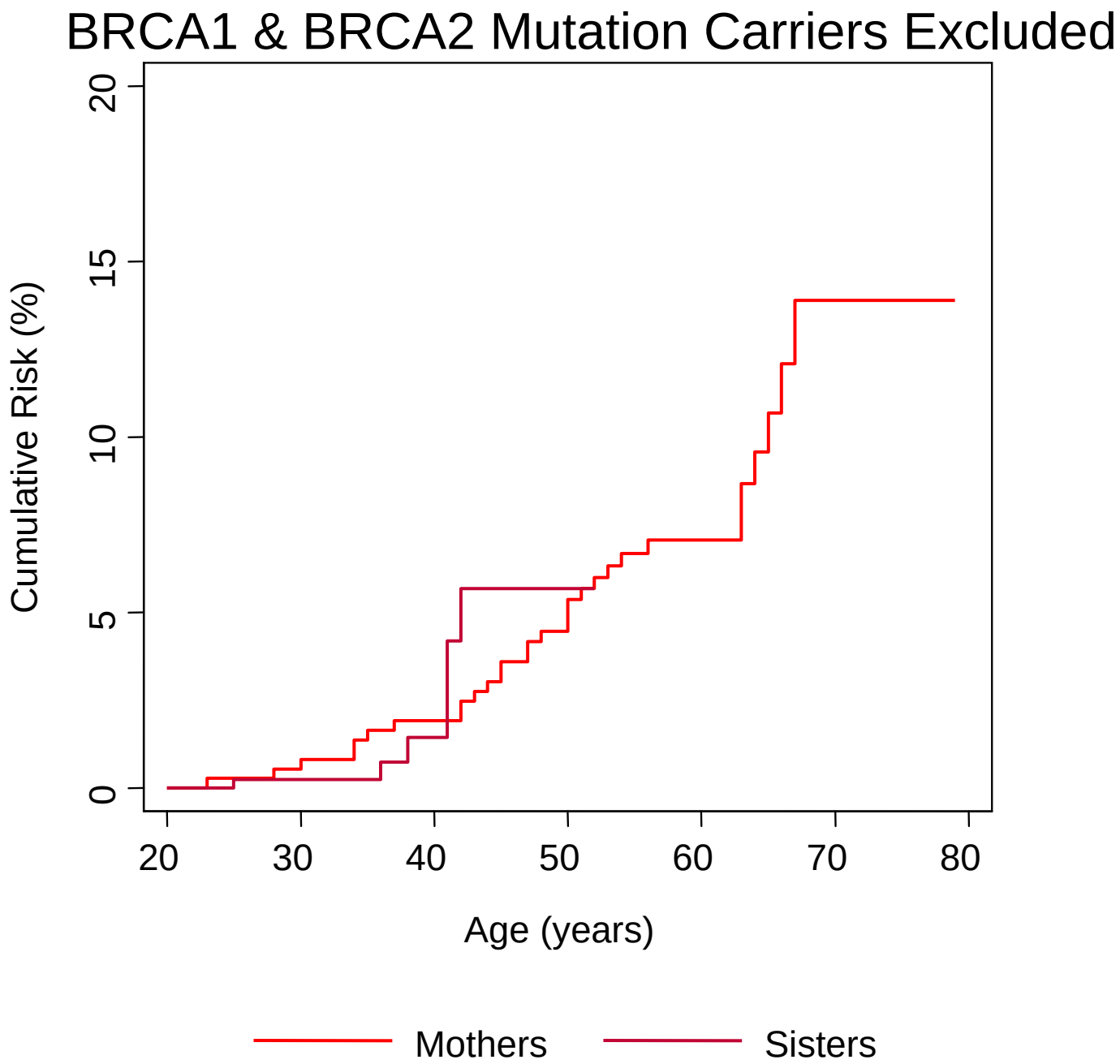
20
Sisters
Mothers
20
30
40
50
60
70
80
Age (years)
Mothers
Sisters


BRCA1 & BRCA2 Mutation Carriers Excluded
Cumulative Risk (%)
0
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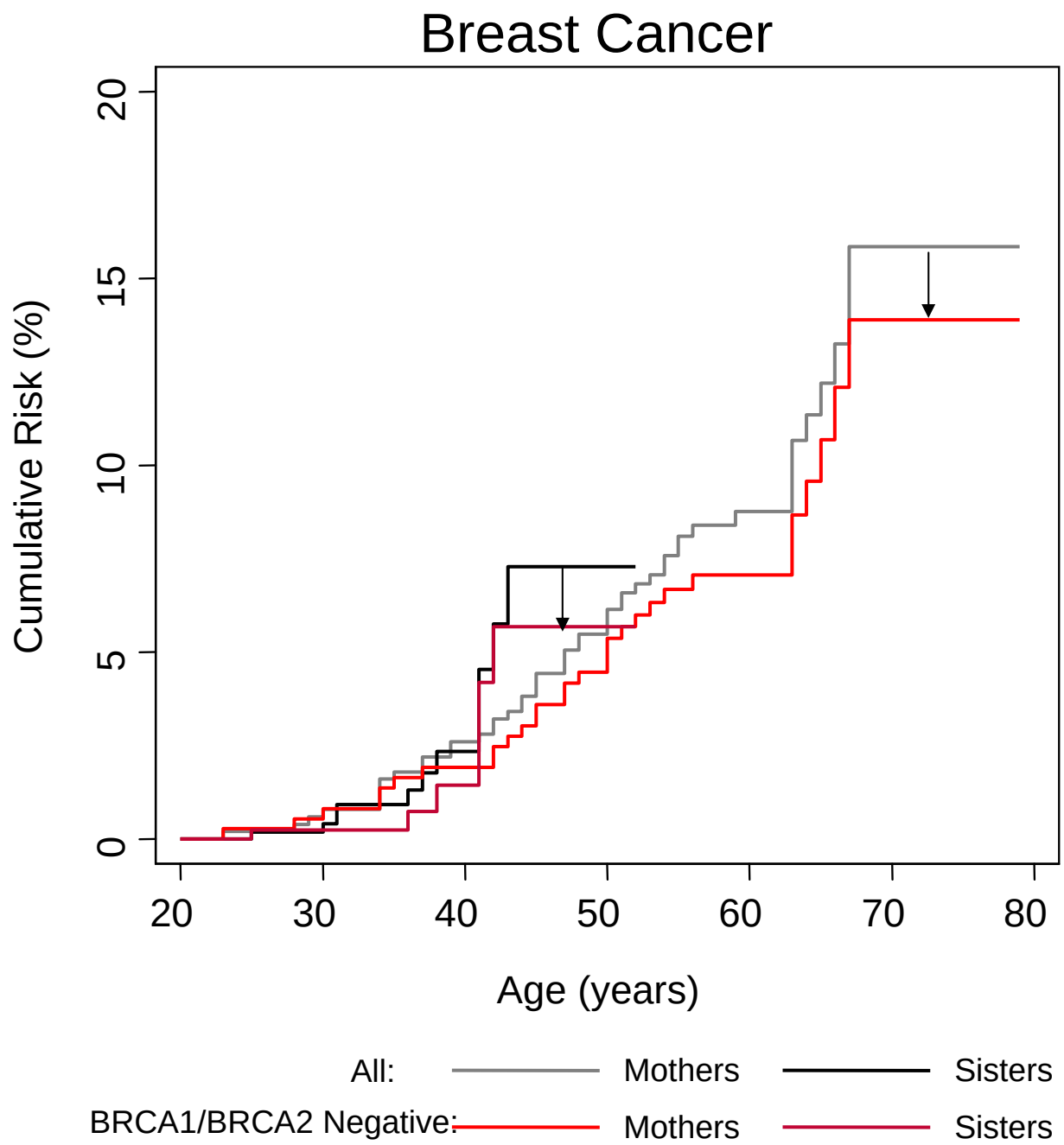
70
80
Age (years)
Mothers
Sisters


Breast Cancer
Cumulative Risk (%)
0
5
10
15
20
20
30
40
50
60
70
80
Age (years)
All:
Mothers
Sisters
BRCA1/BRCA2 Negative:
Mothers
Sisters

Conclusion

- **The majority of familial effects associated with early-onset breast cancer are NOT explained by mutations in *BRCA1 & BRCA2***
- **There are likely to be mutations in genes other than *BRCA1* and *BRAC2* which confer a high risk of breast cancer at a young age**
- **See also Southey et al., 2006**

Steroid Hormone Metabolism Genes

- Breast cancer has an hormonal aetiology
- Many breast cancer risk studies of common polymorphisms in steroid hormone metabolism genes
- “Common disease – common variant” hypothesis

CYP17A1

- ***CYP17A1*** **encodes the enzyme cytochrome P450c17α**
- **Functions at key branch points in steroid hormone biosynthesis in the adrenal gland, ovary and gonads**
- **Polymorphic; in particular, -34T>C studied extensively wrt hormone levels, exogenous hormone use, hormonally-mediated risk factors such as age at menarche, and breast cancer risk**

CYP17A1 -34T>C polymorphism

- We found *evidence* for an effect restricted to early-onset cases with a family history (Spurdle et al., 2000)
- Further studies by us and others have failed to find support for an overall effect (e.g. Ye & Parry, 2002)
- Also failed to replicate published putative gene-environment interactions (e.g. Chang et al, 2005; Einarsdottir et al., 2005)

CYP17A1 -34T>C polymorphism

	Cases	Controls	OR	95% CI
TT	553 (39%)	311 (40%)	1.0	
TC	621 (44%)	364 (46%)	1.00	(0.84-1.19)
CC	230 (16%)	113 (14%)	1.16	(0.91-1.48)
	1,404	788		

CYP17A1 -34T>C polymorphism

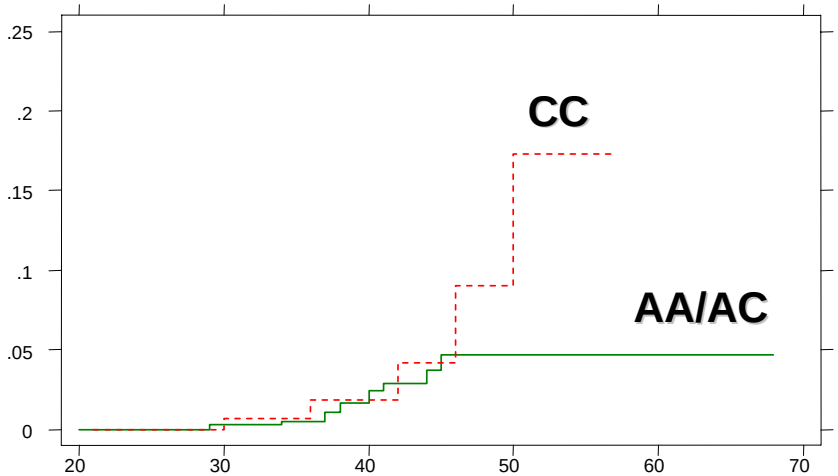
Genotype the 1,903 relatives of the 1,404 cases for whom we have a blood sample

Within-family estimate of average risk associated with CC genotype

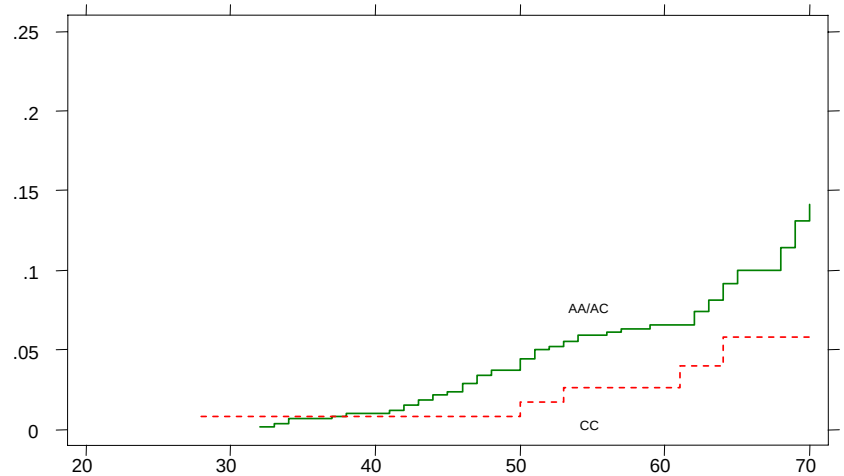
1.20 (95% CI 1.05 to 1.37) (P < 0.01)

What's driving this increased average risk?

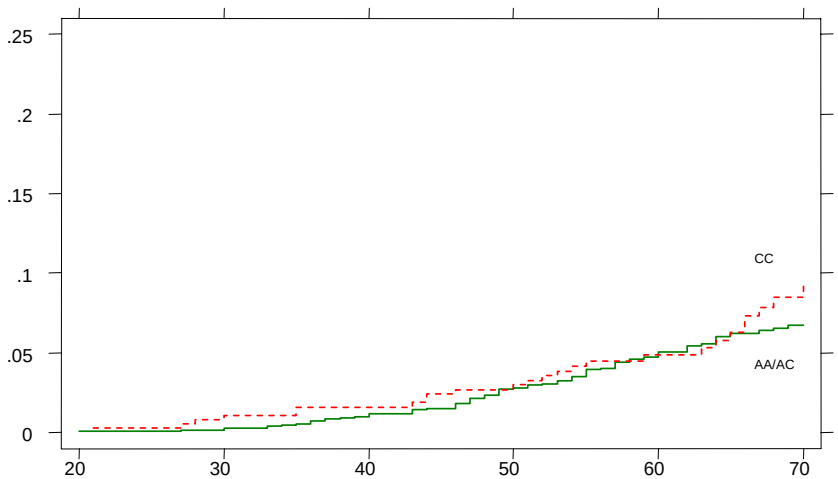
Risks to relatives of cases diagnosed before age 40 by *CYP17A1* genotype



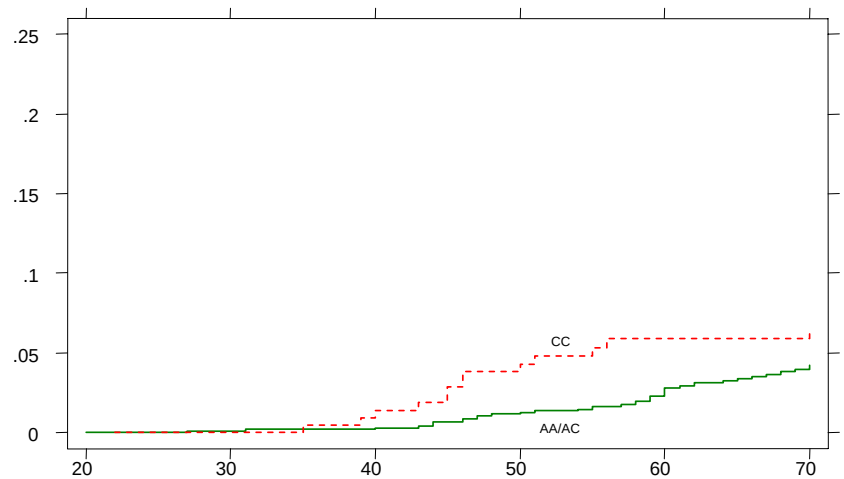
SISTERS



MOTHERS



AUNTS

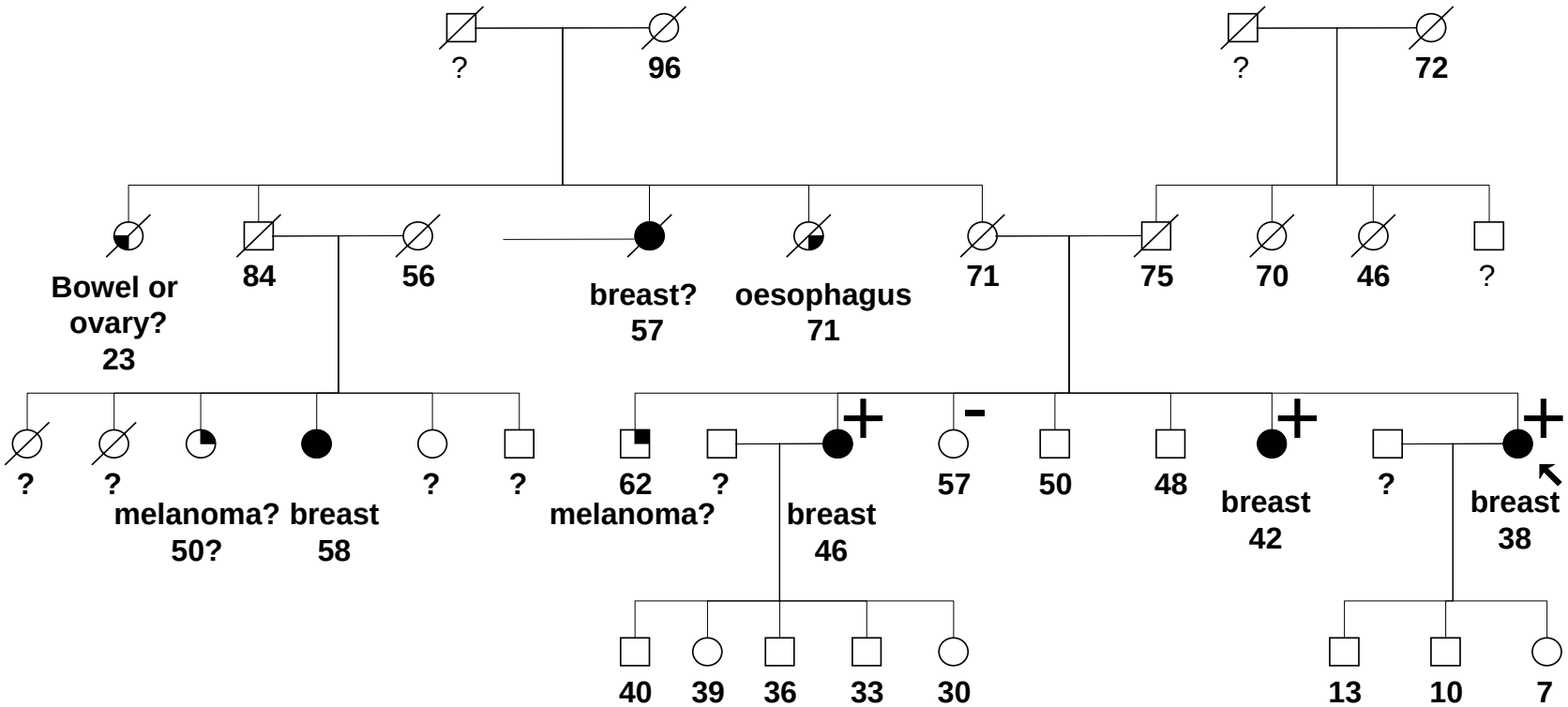


GRANDMOTHERS

Mutation screening for CYP17A1

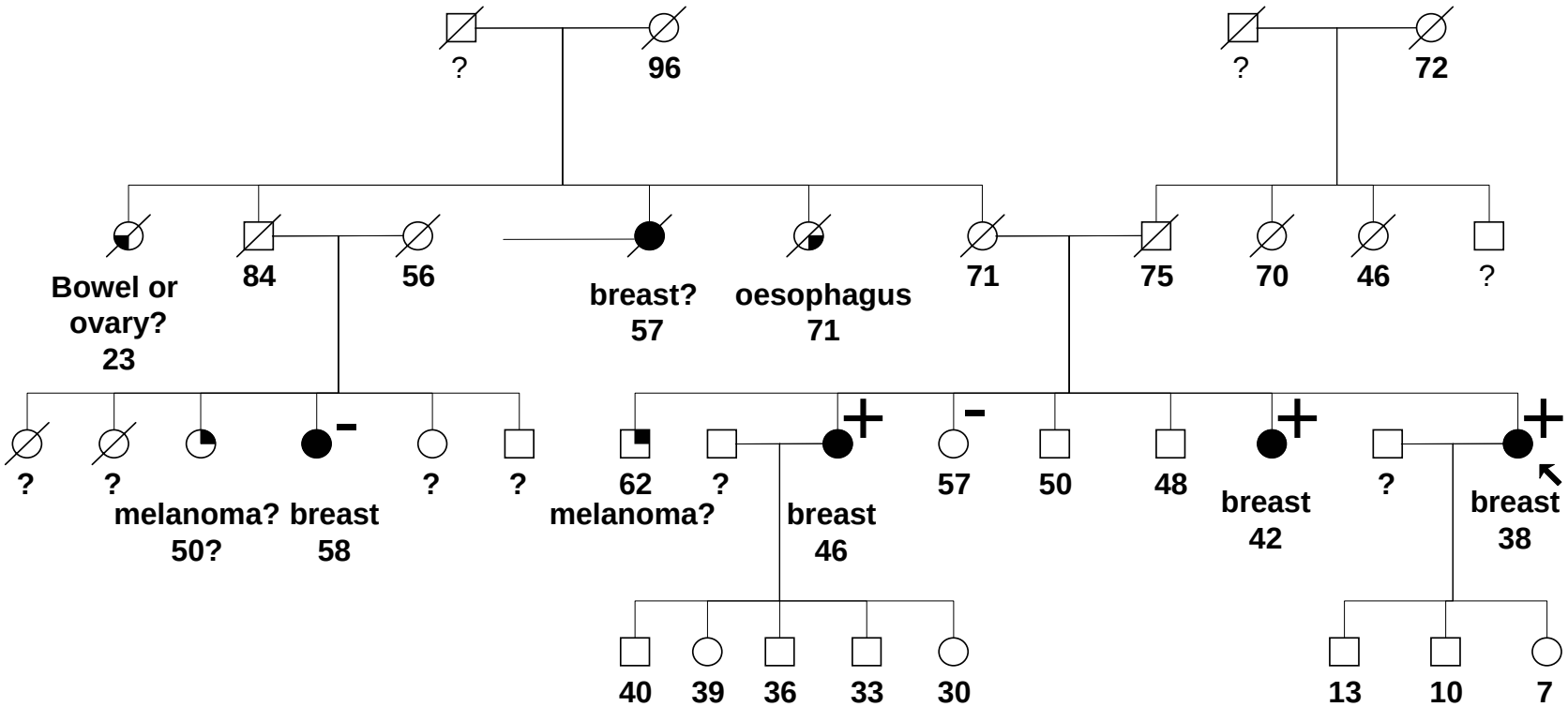
- Screened *CYP17A1* for mutations using denaturing gradient gel electrophoresis (DGGE), starting with cases with affected sisters
- Found R239X protein-truncating mutation in exon 4 in first woman tested
- Mutation carried by two affected sisters, but not by unaffected sister; see pedigree
- Not found in 289 other cases or 788 controls

Hopper et al., *Human Mutation* 2005



+ = protein-truncating mutation in exon 4

Compound heterozygote with this mutation exists and is a pseudohermaphrodite (17α-hydoxylase/17,20-lyase deficiency syndrome)



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CYP17A1 R239X mutation

- Protein-truncating; exon 4
- Compound heterozygote pseudohermaphrodite (Ahlgren et al., 1993)
- 17α-hydoxylase/17,20-lyase deficiency syndrome
- Carried by mother (breast cancer free at 39)

- There is also a founder mutation in *CYP17A1* in a Canadian Mennonite community

Next steps …

- Screen all early-onset cases, starting with those with a strong family history, for mutations in other hormone metabolism genes, in the order ….
- Check putative risk alleles by testing in all cases and controls
- Conduct segregation, Bayesian analyses, LOH and other *in silico* and evolutionary conservation analyses
- Test other multiple-case families
- Do same for other cancers; e.g. prostate

Conclusions

- Common polymorphisms in currently-identified candidate genes may have little if any effect on breast cancer risk, but …
- There may be mutations in hormone-metabolism genes that confer high risk of breast (and other?) cancers at a young age
- It may be possible to discover “high risk genes” using population-based case-control-family studies

Acknowledgements

Epidemiology and Statistical Analyses

Margaret McCredie, Graham Giles, Mark Jenkins, Roger Milne, Gillian Dite

Genetic Analyses

Melissa Southey, Vanessa Hayes, Andrea Tesoriero, Letitia Smith

Co-ordination and Interviewing

Judi Maskiell, Lesley Porter, and many others

Study participants and their relatives

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